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Úloha inkretinových hormonů při vzniku diabetes mellitus 2. typu

The Role of Incretin Hormones in the Development of Type 2 Diabetes Mellitus

Bakalářská práce

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Podpis

Poděkování

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Abstract

Incretins are gut hormones secreted by cells located in the small intestine in response to ingestion of nutrients. The aim of this thesis is to describe their effect on the control of glucose homeostasis. Incretin receptors are widely distributed in multiple tissues and organs suggesting their complex effects including the regulation of glucose homeostasis and food intake by various mechanisms including both peripheral and central effects. Owing to their ability to regulate insulin secretion and glucose metabolism incretins have a potential in the treatment of type 2 diabetes mellitus. Incretin-based therapy strategies are discussed and compared in this thesis. Analogues of glucagon-like peptide-1, one of the incretin hormones, have the ability to lower body weight and therefore are considered as a possible obesity treatment both in patients with and without diabetes. The role of incretins in pathophysiology of obesity and studies carried out in order to evaluate its potential in the treatment of this disease are depicted. The thesis also involves an overview of possible role of incretins in metabolic effects of bariatric surgery.

Keywords: Incretin hormones, GIP, GLP-1, type 2 diabetes mellitus, obesity.

Abstrakt

Inkretiny jsou gastrointestinální hormony vylučované buňkami lokalizovanými v tenkém střevě v závislosti na průchodu potravy střevem. Cílem této práce je popsat jejich vliv na regulaci metabolizmu glukózy. Receptory pro inkretiny se vyskytují v řadě tkání a orgánů včetně pankreatu, jater a centrálního nervového systému, což naznačuje jejich komplexní působení mimo jiné na regulaci glukózové homeostázy a příjmu potravy různými mechanismy zahrnujícími jak působení periferní, tak i centrální. Vzhledem ke schopnosti regulovat koncentrace insulinu a glukózy v krevní plazmě mají inkretiny potenciál v léčbě diabetu mellitu 2. typu. V práci jsou diskutovány a porovnány různé možnosti využití inkretinů v léčbě diabetu. Analoga GLP-1, jednoho z inkretinových hormonů, snižují tělesnou hmotnost, což naznačuje možnost jejich využití i v léčbě obezity, a to i bez přítomnosti diabetu. Popsána je také možná úloha inkretinů v patofyziologii obezity a studie provedené za účelem zhodnocení jejich léčebného potenciálu u nemocných s tímto onemocněním. V práci je dále popsána možná úloha inkretinů při zprostředkování metabolických efektů bariatrické chirurgie.

Klíčová slova: Inkretinové hormony, GIP, GLP-1, diabetes mellitus 2. typu, obezita.

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Introduction

According to the World Health Organization (WHO) publication *Global Status report on noncommunicable diseases* from 2014, 9 % of the world-wide adult population was diagnosed with diabetes with 90 % of them having type 2 [1]. The 7th edition of the *Diabetes Atlas* by International Diabetes Federation (IDF) talks about 318 million people with impaired glucose tolerance that are likely to develop diabetes. Additionally, many people are unaware of their condition because symptoms are not very often as clear as in type 1. The *Diabetes Atlas* claims that up to 1 in 2 adults goes undiagnosed. Further complications include cardiovascular and kidney disease and nerve damage. With rising healthcare expenditures on diabetes patients, the disease is making its impact on economics as well [2].

Type 2 diabetes mellitus (T2DM) is manifested by insulin resistance and β-cell malfunction. Although it can be managed by lifestyle and dietary changes to a certain degree, medication is needed in the majority of cases where these modifications do not lead to successful treatment. Incretins are gut-derived factors that promote insulin secretion when blood glucose levels are high due to an oral intake of nutrients and therefore prevent hyperglycaemia [3, 4]. Incretin response to nutrients is impaired in T2DM patients [5] and its normalization became one of the main targets of novel treatment strategies.

Obesity currently belongs amongst major worldwide health problems. It is defined by body mass index (BMI), derived from weight in relation to height, that is higher than 30 kg/m^2. In 2015, more than 600 million adults were obese according to WHO [6]. Excessive fat accumulation is a potential risk factor and may lead to other illnesses, such as accelerated atherosclerosis, hypertension and diabetes [6]. As origin of obesity is multifactorial, it is targeted by more than one strategy including diet and exercise as well as incretin-based therapy in cases, where change of lifestyle has been of no help. Incretins are associated with weight loss [7-10] and therefore are suitable for managing obesity.

The potential of incretin treatment and its future possibilities are discussed in this thesis.

Molecular Structure and Secretion of Incretins

Glucose-dependent insulinotropic polypeptide (GIP) was the first described incretin hormone. It is a 42-amino acid peptide secreted within the K cells, which are found in the small intestine, specifically in the proximal part [11]. Study on intestinal cell line from transgenic mice, in which GIP promoter has control over human insulin gene expression, showed that GIP expression is regulated by the first 193 base pairs upstream of the transcription initiation site. Transcription factors GATA-4 and ISL-1 appear to control the process as its motifs were found between base pairs −193 and −182 and bases −156 and −151

and their removal resulted in loss of activity by 90 % and 35 %, respectively [12]. The human GIP gene promoter is also regulated by responsive element of cyclic adenosine monophosphate (cAMP) called CRE and c-Jun proteins, responsible for its basal activity [13]. Once the proGIP is expressed, it has to be cleaved to biologically active form by endoproteinase enzymes called proconvertases (PCs), in this case by PC1/3. PC1/3-deficient mice were unable to process proGIP to GIP efficiently. Higher levels of proGIP were not found even if levels of preproGIP were in normal range in comparison with the wild type mice. Increased concentrations of proGIP were not identified probably due to rapid degradation [14]. GIP secretion is nutrient mediated and species dependent. In humans the strongest stimuli are fat followed by carbohydrates, leaving amino acids the weakest ones [15, 16]. GIP fasting levels range around 10 pmol/l and rise to concentrations from 50 to 120 pmol/l after ingestion in healthy subjects [5]. GIP is quickly degraded by enzyme dipeptidyl peptidase-4 (DPP-4) from the N-terminus leaving the majority of GIP present in the system in its biologically inactive form. Whether the origin of GIP is exogenous or endogenous does not matter. Until recently, methods able to distinguish between the two forms of GIP were not yet developed causing artificial results in the actual active levels of the peptide [17]. The half-life of GIP is approximately 4 minutes [18].

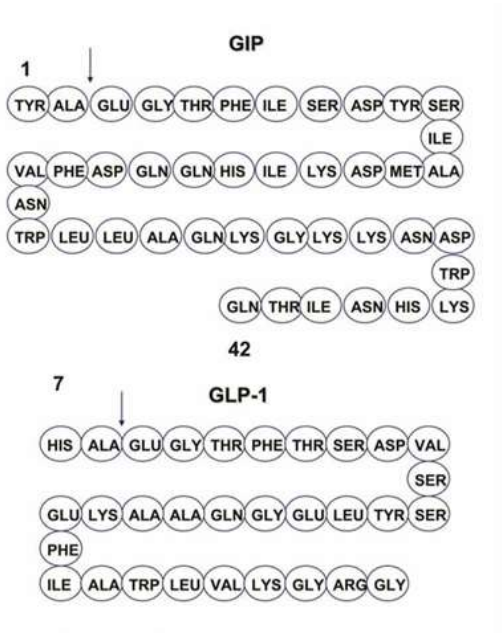

Figure 1 Amino acid sequences of GIP and GLP-1(7–37). Site of cleavage by (DPP-4 is shown by arrow. Adapated from Ranganath LR. *The entero-insular axis: implications for human metabolism.* Clinical Chemistry and Laboratory Medicine: CCLM / FESCC. 2008; Vol. 46 (1), pp. 43-56.

The cloning and the sequencing of the mammalian proglucagon gene led to a discovery of another incretin hormone, glucagon-like peptide-1 (GLP-1). It is a 31-amino acid peptide secreted within the L cells [11]. L cells are found more in the distal ileum than in the duodenum or jejunum in the small intestine. Additionally, cells in the terminal ileum that produce not only GLP-1, but GIP as well were identified. These double-incretin positive cells, so-called K/L cells, vary in expression of the pancreatic and duodenal homebox 1 (PDX-1) [19]. Proglucagon is cleaved from preproglucagon encoded by the GCG gene found on the second chromosome in humans [20]. Wnt signalling pathway via β-catenin (β-cat) and T-cell factor-4 (TCF-4) seems to be the key element in activation of the gene [21]. The reason of insulin ability to stimulate GLP-1 expression is that it encourages the binding of both β-cat and TCF-4 to the promoter [22]. The posttranslational processing of proglucagon is strictly tissue specific and leads to

formation of GLP-1 in L cells as well as GLP-2, oxantomodulin and glicentin. However, in pancreatic α-cells, the resulting peptide is glucagon [23, 24]. PC1 and PC1/3 appear to mediate the cleavage of GLP-1 and GLP-2 from proglucagon as PC1/3-null animals are not able to produce any active forms of these peptides and a case of PC1 human deficiency is consistent with these findings [25, 26]. GLP-1 is originally synthetized as amino-acid polypeptide with a glycine carboxyl at C terminus and needs to be processed to its active form by removing six amino acids. The forms present in the body are GLP-1(7-37) and more abundant GLP-1(7-36)amide [27]. Recently, other shorter forms of GLP-1 with additional biological effects were found. GLP-1(28-36)amide enters glucolipotoxic β-cells and recovers mitochondrial membrane potential. Glucolipotoxicity is caused by higher concentrations of glucose and fatty acids and elevates the possibility of caspase activation which leads to apoptosis. GLP-1(28-36)amide by its actions prevents such state of pancreatic β-cells and thus protects them [28]. GLP-1 secretion in the first phase, right after the food intake but before the nutrients reach small intestine, is driven by a neural reflex and circulation factors, such as vasoactive intestinal peptide and pituary adenylate cyclase-activating peptide [29, 30]. After that it is caused by an interaction of nutrients with the microvilli of L cells in the second phase of the release [31]. GLP-1 fasting levels range from 5 to 10 pmol/l [30] and rise within 15 minutes after ingestion, peak at around 40 to 90 minutes and then reach a plateau in healthy humans [32, 33]. The main stimuli for secretion are carbohydrates and fat, but only carbohydrates stimulates both GIP and GLP-1-mediated insulin release [34]. Somatostatin inhibits the secretion, suggesting the existence of a negative local feedback mechanism [35]. Specific G-protein coupled receptors are obligatory for normal occurring secretion, specifically GPR120 [36], GPR119 [37] and GPR40 [38]. Both exogenous and endogenous GLP-1 are rapidly metabolized and inactivated by the enzyme DPP-4. DPP-4 cleaves GLP-1 at the NH_2 terminus [39] making the half-life of the intact and biologically active molecule of 2 to 4 minutes in plasma [40, 41].

Signalling Mechanisms of Incretin Effect

Both GIP and GLP-1 act via G-protein-coupled receptors present in pancreatic islets, heart and nervous system. Besides that, GIPRs can be found in adipose tissue and GLP-1Rs in kidneys and lungs. Glucose dependent insulin secretion from pancreatic β cells is regulated by intracellular signals in order to maintain glucose homeostasis. Entry of glucose via facilitated diffusion leads to production of ATP and closure of ATP-sensitive K^+ channels. The closure affects membrane potential and causes opening of Ca^{2+} channels that are voltage-gated and the influx of Ca^{2+} ions promotes exocytosis of insulin granules [42, 43]. Signalling involves two separate mechanisms. The first one mediated by cAMP-binding protein

kinase A (PKA) and the other by cAMP-regulated guanine nucleotide exchange factor 1 and 2 (*Epac*) [44]. GIP alongside stimulating cAMP signalling causes PKA-dependent endocytosis of voltage-gated K^+ (K_V) channels. GIP in GIPR expressing cells decreased peak ionic current amplitude of K_V by activation of PKA that led to phosphorylation-dependent endocytosis of the channels. A mutant form of the cell line resistant to PKA phosphorylation showed lower glucose-induced insulin secretion [45].

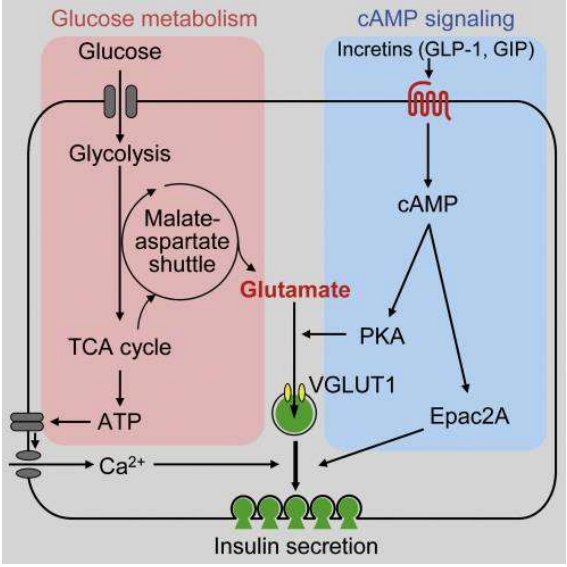

Figure 2. Incretin signalling pathways. Adapted from Gheni G. *Glutamate acts as a key signal linking glucose metabolism to incretin/cAMP action to amplify insulin secretion*. 2014; *Cell rep.*, 9(2): 661–673.

Incretins affect β cells only when glucose concentrations are higher. The key element connecting glucose metabolism and insulin release seems to be cytosolic glutamate from malate-aspartate shuttle. The glutamate is derived from α-ketoglutarate through the shuttle in response to glucose. A study of insulin-responsive and unresponsive β-cell lines showed that concentrations of glucose 6-phosphate, fructose 6-phosphate, fructose 1,6-bisphosphate, nicotinamide adenine dinucleotide phosphate (NADPH), glutamate and aspartate were significantly higher in responsive cells. This suggests increased activity of malate-aspartate shuttle. The incretin-induced insulin secretion is abolished by the shuttle inhibitor and in knockdown mice of the aspartate aminotransferases or aspartate/glutamate carrier whereas glucose-induced insulin secretion is not affected in any way. Further experiments proved that glutamate uptake into insulin granules through vesicular glutamate transporter 1 (VGLUT1) is necessary for the exocytosis in a PKA-dependent manner by capacitance measurements, internal reflection fluorescence microscopy analysis and VGLUT1 knockout and knockdown [46].

GLP-1 is also responsible for replenishing insulin stores by the use of cAMP-mediated pro-insulin gene transcription. During 24h incubation the insulin mRNA concentrations were three-fold higher when GLP-1 was added than in standard cells. Other increased mRNA levels were not present as an evidence to the specific actions of GLP-1 [47]. GLP-1 acts via stimulation of transcription factor of pancreatic and duodenal homebox 1 (PDX-1) gene synthesis which then binds to insulin gene promoter. PDX-1 mRNA levels were proved to be notably higher upon treatment of insulinoma cells with GLP-1. The PDX-1 protein expression was therefore 1.6-fold increased. GLP-1 even upregulates the binding to the A1

element of the insulin promoter. Glucose had an additive effect on the whole process [48]. Furthermore, by PDX-1 regulation, GLP-1 helps to sustain β-cell mass as it is essential for its proliferation and cytoprotection. The β-cell PDX-$1^{-/-}$ mice exhibit not only deterioration in insulin secretion but fail to produce anti-apoptotic and pro-proliferative factors upon GLP-1R activation [49].

Physiologic Effect of Incretins

Incretins mainly serve to maintain glucose homeostasis after meal ingestion and thus prevent hyperglycaemia which leads to health complications associated with diabetes. Older studies claim essential role of GIP for sustained blood glucose levels as $GIPR^{-/-}$ mice exhibited glucose intolerance and impaired insulin secretion after oral administration. Fasting levels of glucose did not differ much between $GIPR^{-/-}$ and $GIPR^{+/+}$ but peak levels of glucose were delayed and significantly higher in -/- (406 ± 28 mg/dl at 20 min vs. 312 ± 24 mg/dl at 10 min). The insulin concentrations were on the contrary lower (641 ± 54 pg/ml vs. 1,101 ± 68 pg/ml). Moreover, compensatory increased levels of insulin in -/- high-fat diet, such as those in +/+, were not visible suggesting a defective entero-insular axis [3]. In more recent studies also using GIPR knockout mice, the alterations were not as significant and deficient animals suffered from only moderate glucose metabolism impairment. This suggests an existence of compensatory mechanism. GLP-1-induced insulin release was more prominent under these circumstances and islets had different topography [50]. Rats pretreated with GIPR antibodies and unable to mediate GIP signalling, while the GLP-1 signalling remained unaltered, showed delayed and 35 % lower insulin response to glucose [51]. GIP also has a glucagonotropic effect and dose-dependently enhances glucagon secretion from α cells at euglycaemia. Glucagon is responsible for glucose release into blood in order to prevent low levels of glucose in blood. This was proved in a study with 10 healthy subjects receiving variable doses of GIP given intravenously. The glucagon levels differed by 1.4 ± 0.5, 2.4 ± 0.5, and 3.4 ± 0.8 pmol/l for 7, 20, and 60 pmol GIP/kg, respectively [52]. GLP-1, likewise GIP, has an insulinotropic effect. The receptor antagonist exendin(9-39)amide injected into anesthetised rats decreased incretin-induced insulin release by 60 % and thus increased circulating glucose [53]. Disruption in GLP-1R resulted in lowered insulin levels as well [4]. Similarly, as it does in case of GIPR disruption, when GLP1-R is not functional, a compensatory mechanism takes over. Serum GIP levels are increased in GLP-1R knockout mice after an oral glucose challenge (369 ± 40 vs. 236 ± 28 pmol/l in wild type mice) pointing out an upregulation of GIP [54]. Extended infusions of GLP-1, in addition to promotion of insulin secretion, shifted the response to earlier release. The previously stated was concluded by a study with nine nondiabetic volunteers that underwent three infusions. The first without GLP-1, the second being

acute and the third continuing for 3 hours. Fasting plasma glucose was significantly increased when the infusion was extended and shifted to the first 10 minutes. The effect on β cells is apparently time dependant [55]. GLP-1 also modifies glucagon release. As expected, it does so in an opposite manner than GIP. It supresses the release as seen in an experiment using duodenal glucose perfusion accompanied by intravenously given GLP-1 antagonist exendin-(9-39)amide. Prior the administration of the antagonist glucagon levels gradually lowered during the glucose perfusion. When exendin-(9-39)amide was added at glucose perfusion rate 1 kcal/min, mimicking the fasting state, it did not have any significant effect. However, when the rate was 2.5 kcal/min, the decrease of glucagon was completely inhibited by the antagonist. GLP-1 therefore seems to abolish postprandial glucagon secretion [56].

Apart from promoting insulin release from β-cells, GLP-1 also slows down gastric emptying and allows better nutrition ingestion in the small intestine. The extent of decreased rate of the emptying is dependent on the load of glucose. The higher the glucose load is, the more prolonged the emptying. The deceleration occurred even with GLP-1 dose as small as 0.4 pmol $\cdot$ kg^{-1} $\cdot$ min^{-1} and gastric contents stayed at ± 250 ml of volume after 240 min subsequently the meal [57]. The relationship between caloric dose and deceleration is nonlinear, with decreased at first and then accelerated rate of emptying in the end of the experiment [15]. Postprandial plasma levels of GLP-1 decreases the amount of antro-duodenal contractions and its amplitudes and inhibits antral wave propagation in the interdigestive state. The tonic and phasic motility of the pylorus was on the other hand elevated [56, 58]. GIP, unlikely of GLP-1, does not alter the emptying [59].

The slowing of gastric motility is diminished when vagal afferent neurons are eliminated, confirming that GLP-1 also acts as a gut-brain factor [60]. In accordance with this is the finding that GLP-1-induced insulin release is inhibited by ganglionic blockade and suggest inclusion of a nervous mechanism, most likely via the vagus nerve [61]. GLP-1 receptors are definitely present in the brain and incretins were proved to be able to pass the blood-brain barrier. GLP-1R mRNA was confirmed by *in situ* hybridization to be present in many parts of the brain, including areas involved in the control of food intake, reward processing and motivated behaviour, such as hypothalamus, solitary nucleus (NTS) and area postrema (AP) of the brainstem, dorsal striatum, nucleus accumbens alongside the diagonal band of Broca, bed nucleus of the stria terminalis, substantia innominate, amygdala, preoptic area, several parts of the midbrain, including locus coerulus, A1/C1. The hippocampus and thalamus contained low amounts of GLP-1R mRNA. The staining was carried out using the brain of a primate *Macaca mulatta* [62]. The expression of GLP-1R in the hypothalamus explains the ability of GLP-1 to reduce food intake as one of

the nuclei controls satiety. The lateral and third ventricular and intracerebral injection of GLP-1 significantly supress caloric intake in rodents. Additionally, injection of GLP-1R antagonist, exendin(9-39) amide, promoted food intake, especially with three repetitive injections, which increased intake by 300 % [63]. Satiated vagotomised and sham-vagotomised rats were injected with GLP-1. GLP-1 significantly reduced food intake in sham-vagotomised but bilateral sub-diaphragmatic total truncal vagotomy and brainstem–hypothalamic pathway transectioning blocked its effect suggesting important role of the vagal pathway in activation of ARC neuronal circuits [64]. The hypothalamic and brainstem circuits do not seem to be the only ones responsible for GLP-1 effects. The mesolimbic reward system also plays a role in food intake. Infusions of exendin-4 in ventral tegmental area and NAc were given to mice prior behavioural testing. A dose-dependent suppression of motivated behaviour was achieved. This was measured by a standardised test, where rats had to perform a task (press a lever) for a reward. It was also evident by the indication that rats did not spend that great deal of time in places associated with the reward as controls [65]. GIP also plays a role in controlling food intake. Six healthy lean male subjects were given an infusion of GIP for 4 hours and their calorie intake and energy expenditure via indirect calorimetry was measured afterwards. Subjects reported higher hunger scores and a decreased energy expenditure when compared with placebo [66].

Another physiological effect of GLP-1 is that on β-cell mass differentiation, proliferation and glucose sensitivity. GLP-1 improves glucose competence of glucose-resistant-β cells by inhibition of ATP-sensitive potassium channels [67]. A number of experiments were held on human pancreatic islets and showed that islets treated with GLP-1 were able to hold their 3D structure up to the day 5 with only a 15 % reduction of mass in comparison to 45 % reduction in control samples. Less apoptotic changes to the cell nucleus were also visible due to strong stimulation of antiapoptotic molecule B-cell lymphoma 2 (Bcl-2) and inhibition of proapoptotic caspase-3 by GLP-1 [68]. Both GLP-1 and GIP act via activation of protein kinase A/cAMP response element binding protein (PKA/CREB), mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase/protein kinase B (PI3-kinase/PKB) pathways and Ca^{2+} signalling. GLP-1 is more dependent on PI3-kinase activation. The main target of incretins is cyclin D1, a co-factor of cyclin-dependent kinases that by its activity release E2F transcription factors promoting many S-phase genes [69]. GLP-1 proved successful in increasing incretin-induced insulin release in islets and pancreas transplant recipients. Eleven subjects underwent testing with GLP-1 infusions and resulted with higher insulin level after 30 minutes compared to placebo (21.6 ± 7.8 vs. 7.4 ± 3.5 μU/ml in the islet group and 19.3 ± 4.8 vs. 10.2 ± 1.5 μU/ml in the pancreas group). Also second-phase insulin was augmented in both groups in comparison to placebo (129.9 ± 89.8 vs. 32.3 ± 24.2 μU/ml in the islet group and 298 ± 51 *vs*.

45.5 ± 12.4 μU/ml in the pancreas group). GLP-1 thus seems to be a potent key to the treatment of patients with either islet or pancreas transplant [70].

Additionally, GLP-1 may have another extrapancreatic physiological actions such as acutely increasing blood pressure and heart rate [71] or affecting rat behaviour, making experimental animals more exploratory and physically active [72].

Pathophysiology of Type 2 Diabetes Mellitus

The incretin response in type 2 diabetes is reduced in comparison with healthy subjects, especially in the late phase of the meal response [5]. This however, may be be rather a consequence of the condition than a primary cause, at least in some patients. In a study with three different groups of subjects, chronic pancreatitis and diabetes patients plus healthy subjects, all patients on antidiabetic drugs and/or diet had well preserved glucose homeostasis, suggesting sustained amount of active β-cells. Nonetheless, the incretin effect was significantly reduced in diabetes patients in contrast with pancreatitis patients who had similar incretin effect as that of healthy subjects. A reduction of β cell mass is not likely to be an explanation as insulin responses were similar during glucose infusions in all groups. GLP-1 and GIP levels were higher in diabetic patients when measured after oral glucose challenge pointing to a dysfunction of β-cell responses [73].

As for GIP, its lack of effect on β cells is attributed to GIPR desensitisation and/or down-regulation [74]. GLP-1 infusions were established to be very beneficial in restoring glucose homeostasis in T2DM. Intravenous infusion of GLP-1 managed to lower both fasting and mean 24-h plasma glucose and increase insulin levels. Plasma glucose levels decreased from 14.1 ± 0.9 to 12.2 ±0.7 mmol/l and from 15.4 ± 1 mmol/l to 13 ± 1 mmol/l for fasting and mean levels, respectively. Levels of insulin increased from 82 ± 20 to 107 ± 18 pmol/l and from 189 ± 40 to 224 ± 48 for fasting and mean levels, respectively [75]. Continuous GLP-1 infusions for 6 weeks significantly decreased plasma concentrations of glycated haemoglobin A1c and insulin measured at lower plasma glucose concentration suggested improved β-cell sensitivity, reinforced also by improved insulinogenic index [76].

Hyperglycaemia, accompanying T2DM, causes further damage of β-cell mass by glucotoxicity and glucolipotoxicity [77]. Furthermore, T2DM patients have increased islet amyloid deposition. Human pancreas sections from 29 T2DM and 39 healthy subjects were detected for visualized amyloid and immunolabled insulin. An inverse correlation between β-cell volume and amyloid deposits was found.

Islets with bigger amount of amyloid exhibited strong signs of progressing β-cell apoptosis indicating an association amongst amyloid deposition and β-cell deterioration [78].

Another factor adding to the pathophysiology of T2DM is more rapid gastric emptying in some diabetic patients, which can be also modified by GLP-1. Study on 18 subjects with 9 of them being diagnosed with T2DM confirmed increased rate of emptying. The half-emptying time was measured 33.6 min for diabetic patients vs. 64.6 min for healthy controls. The most noteworthy difference occurred at 75 min. The average rate of emptying was 3.3 kcal/min vs. 1.6 kcal/min for diabetic and healthy subjects, respectively [79].

Incretin-based Therapy

The main targets of incretin-based therapy are to improve incretin effect and the state of β-cell mass, promote weight loss, slow gastric emptying and decrease hyperglucagonaemia. Two strategies in incretin treatment are used, incretin mimetics and DPP-4 inhibitors. Both have its pros and cons that are about to be discussed.

Glucagon-like Peptide-1 Mimetics

Glucagon-like peptide-1 mimetics are molecules that share a certain degree of similarity in amino acid structure as GLP-1 and possess the ability to activate GLP-1R without being degraded by DPP-4 too rapidly. GLP-1 mimetics have to be administered subcutaneously.

The first GLP-1 agonist was isolated from the venom of a Gila lizard *Heloderma suspectum* with a 53 % homology to GLP-1 and has a glycine as a second amino acid [80]. Exenatide at doses from 0.02 to 0.2 μg/kg provided dose-dependent antidiabetic activity, reduced postprandial plasma glucose and glucagon and slowed gastric emptying. It is detectable in plasma after 15 minutes of injection and persists in active form for as long as 15 hours if the dose 0.2 μg/kg or higher [81]. Exenatide administered twice daily was examined in a triple-blind, placebo controlled, 30-week study. At the end, HbA_{1C} levels, used to assess diabetes compensation, dropped by -0.86 ± 0.11 % in the 10 μg group and by -0.46 ± 0.12 % in the 5 μg group. On the contrary, the placebo group showed an increase in HbA_{1C} of 0.12 ± 0.09 %. 41.3 % and 32.6 % of subjects reached target HbA_{1C} levels of -7 % or less in 10 and 5 μg group, respectively. Body weight was decreased by week 30. The group with dose of 10 μg of exenatide lost 1.6 ± 0.3 kg and group with 5 μg lost 0.9 ± 0.3 kg relative to baseline values. The loss appeared to be progressive and did not seem to reach plateau by the end of the study. The treatment was very well tolerated with no adverse events than mild nausea in the first few weeks [7]. An encapsulation of

exenatide into poly-(D,L-lactide-co-glycolide) (PLG) microspheres enables exenatide to be released into the blood slowly and for longer period of time. Therefore, it can be administered less frequently. The first 1 % is released in the first few hours. The therapeutic dose is reached by week 2 and persists for 6 to 7 weeks. The PLG matrix is hydrolysed to lactic and glycolic acid and then to carbon dioxide and water [82]. In a 30-week randomised study 2 mg exenatide once weekly (OW) was compared with 10 μg twice daily (TD). The study involved 295 patients with T2DM with HbA_{1C} 8.3 % and average duration of the condition of 6.7 years. At the end of the study, patients in both groups showed significant improvements with OW group resulting in greater HbA_{1C} reductions than TD group (-0.54 % vs. -0.12 % for OW vs. TD, respectively). More OW patients achieved target HbA_{1C} levels of -7 % or less (77 % vs. 61 % for OW vs. TD, respectively) and 25 % even achieved values of -6 % or less. Exenatide OW showed significant decrease of postprandial glucose levels but did not affect gastric emptying as much as exenatide TD. Weight loss was observed even in patients with no reported case of nausea. A mild intensity nausea occurred less in OW group. Anti-exenatide antibodies were of higher levels with OW administration but still of too low titre to affect the treatment [8]. Due to increased stability, exenatide is cleared only by glomerular filtration and does not produce any metabolites during its clearance from the organism [83].

Liraglutide differs from GLP-1 by arginine at position 28 and by C16 palmitic acid attached to K20 by glutamic acid spacer. Its pharmacokinetics and pharmacodynamics was examined in a 52-week long study involving patients in the early stages of the disease or treated by diet only that were given once daily subcutaneous injection of 1.2 mg or 1.8 mg of liraglutide. HbA_{1C} dropped by -0.62 % and -0.33 % for 1.8 mg and 1.2 mg dose, respectively. At week 52, 43 % and 51 % of the subjects reached the target HbA_{1C} levels of 7 % or less in 1.2 mg and 1.8 mg group, respectively. More patients in the group, that was treated by diet only prior the study, achieved the target levels. All patients lost weight in the first 16 weeks and it remained the same for the whole 52-week period. No cases of severe side effects were reported. The only complications that occurred were mild nausea [10]. In a comparative study of liraglutide once a day and exenatide twice daily, liraglutide exhibited better efficacy in improvement of diabetes compensation and was better tolerated [84].

Lixisenatide, approved by the European Medicines Agency in 2013 and tested in a 28-day long study in which 77 patients on metformin received 10 μg of the drug. Mean HbA_{1C} was reduced from 7.2 % to 6.9 %. Weight dropped by 1.6 kg. Incidence of adverse events added up to 55 % with gastrointestinal disorders being the most frequent ones [9].

Dulaglutide is another GLP-1 dimer but linked to human IgG4-Fc heavy chain and has three amino acid modifications. It is administered once weekly. Changes to HbA_{1C} were -1.5 % to baseline after 26 weeks on 1.5 mg of the drug [85].

DPP-4 Inhibitors

DPP-4 inhibitors inhibit the action of an enzyme DPP-4 and thus lower its effect on incretins enabling them to act over a longer period of time. The concentration of incretin hormones is consequently higher. Unlike GLP-1 mimetics DPP-4 inhibitors are oral agents and cannot alter gastric emptying and have no effect on preserving β cell mass or controlling appetite [86-90].

Sitagliptin is the first to be used DPP-4 inhibitor. It was tested in a study with 521 patients who were receiving 100 or 200 mg once daily for 18 weeks. By the end of the study HbA_{1C} decreased by -0.6 % and by 0.48 % with dose of 100 and 200 mg, respectively. More patients achieved HbA_{1C} target levels of 7 % or less in the group that was given 100 mg than those with 200 mg (35.8 % vs. 28.6 %). No adverse experiences were reported [91]. Longer study with duration for 52 weeks confirmed the findings and additionally compared sitagliptin to glipizide treatment. The trial involved 1172 patients receiving 100 mg of sitagliptin or 5 mg of glipizide. HbA_{1C} levels decreased by -0.67 % in both groups. The HbA_{1C} target levels of 7 % or less were reached by 63 % in sitagliptin group vs. 59 % in glipizide group. Sitagliptin promoted weight loss of 1.5 kg in comparison with glizipide that led to weight gain of 1.1 kg. Sitagliptin treatment also had much lower frequency of hypoglycaemia (5 % vs. 32 % for glizipide) [86].

Vildagliptin was examined in a 24-week, placebo-controlled study involving 296 patients inadequately treated by insulin receiving either 50 mg of vildagliptin twice daily or placebo. The mean HbA_1C was reduced by -0.5 ± 0.1 % and the older patients (≥ 65 years) exhibited higher reductions of -0.7 ± 0.1 %. No severe adverse events were reported [87].

Efficacy of saxagliptin was examiend in a 104-week randomised, double-blind study and compared with sulphonylurea glipizide as add-on therapy to metformin. The decrease from baseline in HbA_{1c} at the end of the study was −0.41 % vs. −0.35 % with saxagliptin and glipizide, respectively, with 23 % of patients in each treatment group achieving HbA_{1c} levels <7 %. Weight loss of −1.5 kg was achieved with saxagliptin, while glipizide caused weight gain of 1.3 kg. More patients reported hypoglycaemia in the glipizide (38.4 %) than in saxagliptin group (3.5 %). Overall, the treatment was well tolerated [88].

Linagliptin was proved to be effective and safe treatment for patients who do not reach HbA_{1c} goals with metformin or pioglitazone in a randomized, double-blind, placebo-controlled study comprising

of 272 patients. Subjects received 5 mg of linagliptin once daily as add-on to metformin and pioglitazone for 24 weeks. The change in HbA_{1C} from baseline was –0.13 %. The HbA_{1C} target levels of ≤7 % achieved 32.4 % of patients. Fasting plasma glucose decreased by –0.57 mmol/l. The incidence of serious adverse events was 2.2 %. Hypoglycaemia reports made up to 5.5 % [89].

Alogliptin demonstrated its efficacy in a retrospective observational study including cases of 303 patients. The reduction of mean HbA_{1c} was from 7.37 % to 6.78 % after 6 months of treatment and to 6.83 % after 12 months. The percentage of subjects achieving HbA_{1C} target levels of ≤7 % was 63.3 % after a year. Additionally, alogliptin decreased the levels total cholesterol and low-density lipoprotein cholesterol, suggesting lipid-lowering effect. As serum creatinine was increased and estimated glomerular filtration increased, alogliptin could also have influenced kidney function. Twelve adverse events were reported with half of them being constipation and only two hypoglycaemia [90].

Comparison of Efficacy

Both incretin mimetics and DPP-4 inhibitors have been thoroughly studied. The main differences between the two main categories are that DPP-4 unlike GLP-1 does not provide significant weight loss or inhibition of food intake. Its advantage is once-daily oral dosing. GLP-1 agonists are administered subcutaneously once or twice-daily or once-weekly [92-94].

DURATION-4 was a 26-week, double-blind, placebo-controlled study comparing apart from others the effect of exenatide once weekly (EQW) versus sitagliptin as a monotherapy in drug-naive T2DM patients. Exenatide showed better improvement in glucose control than sitagliptin as measured by HbA_{1C} levels. Furthermore, weight loss achieved by EQW treatment was more significant. The rate of adverse events, such as mild hypoglycaemia or nausea, was low in both treatment groups [94]. The longest study so far, 1860-Lira-DPP-4, regarding this topic compared 1.2 and 1.8 mg/day liraglutide against 100 mg/day sitagliptin, both in combination with metformin. It was a one-year, open-label, parallel-group study comprising of 665 T2DM patients. Liraglutide resulted in a better glucose control, more pronounced decrease in HbA_{1C} (by −1.29 % and −1.51 %, −0.88 % for 1.2 and 1.8 liraglutide and sitagliptin, respectively) and in fasting plasma glucose. Also body weight dropped more in liraglutide than in sitaglitpin group (by −2.78 kg and −3.68 kg vs. −1.16 kg, respectively) as seen in fig. 3. On the other hand, adverse events were more frequent in liraglutide patients who experienced gastrointestinal side effects, most frequently nausea, but mostly in the first few weeks of the study only [92].

Meta-analysis of incretin-based therapies compared different studies and its results in mean changes in HbA_{1C}, also adjusted for differences in baseline. Mean [95 % CI] changes were: exenatide twice daily, −1.08 [−1.22 to −0.94]; exenatide once weekly, −1.54 [−1.73 to −1.36]; liraglutide once daily, −1.22 [−1.39 to −1.05]; alogliptin, −0.70 [−0.90 to −0.50]; linagliptin, −0.60 [−0.80 to −0.40]; saxagliptin, −0.71 [−0.89 to −0.54]; sitagliptin, −0.70 [−0.78 to −0.63]; and vildagliptin, −0.98 [−1.46 to −0.52]). Differences between GLP-1 mimetics and DPP-4 inhibitors were: exenatide twice daily, −1.05 [−1.19 to −0.92]; exenatide once weekly, −1.59 [−1.70 to −1.48]; and liraglutide once daily, −1.21 [−1.35 to −1.06] versus alogliptin, −0.65 [−0.80 to −0.50]; linagliptin, −0.61 [−0.75 to −0.46]; saxagliptin, −0.68 [−0.78 to −0.57]; sitagliptin, −0.67 [−0.75 to −0.60]; and vildagliptin, −0.98 [−1.37 to −0.59]) [93]. Results are seen in figure 3.

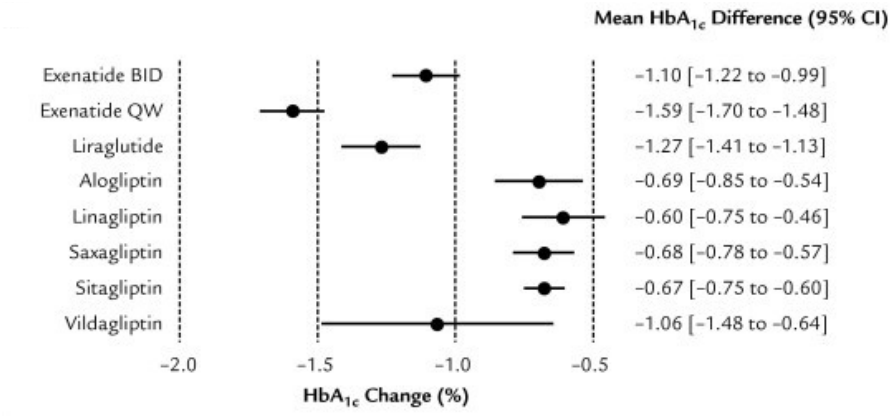

Figure 3 Overall mean changes from baseline in HbA_{1c} with the use of GLP-1 mimetics or DPP-4 inhibitors at the highest maintenance doses evaluated. Adapted from Aroda VR, Henry RR, Han J, Huang W, DeYoung MB, Darsow T, Hoogwerf BJ: *Efficacy of GLP-1 receptor agonists and DPP-4 inhibitors: meta-analysis and systematic review*. Clin Ther 2012, 34(6):1247-1258.e1222.

GLP-1 mimetics as well as DPP-4 inhibitors have antidiabetic effect. However, GLP-1 mimetics led to superior results in both studies suggesting it a better option for T2DM. Most importantly, the aim and intensity of glycaemic control should be always individualized for every patient in order for the treatment to be successful [92-94].

Side Effects

Incretins are linked to side effects affecting gastrointestinal tract. Nausea and vomiting belong among the most frequent [7-9]. Some cases of hypoglycaemia were also reported [86].

As to more severe side effects, acute pancreatitis have been discussed [95]. Recent nationwide study using the medical database of Denmark did not prove an increased risk of pancreatitis. It used data from 12 868 patients hospitalized for acute pancreatitis between years 2005 and 2012 and 128 680 control subjects and searched for an association among the condition and antihyperglycaemic drugs. No link was found despite the fact of overall increased risk of pancreatitis in diabetic patients. The adjusted

odd ratio for incretin users plus other antidiabetic drugs was 0.97. The higher likelihood was caused by underlying diabetes rather than by a drug effect [96].

Questions concerning an increased risk of developing thyroid cancer in incretin patients have been raised in several studies [95] and it seams unlikely in humans in contrast to mice as in contrast to rodents no GLP-1 receptors are present on human C-cells [97].

Novel Strategies Under Development

As GLP-1 mimetics cannot yet be administered in a different way than by subcutaneous injections, orally given agonists are under development. The D-ala^2-GLP-1 has a prolonged therapeutic action due to D orientation of alanine on the second position, as DPP-4 is responsible for L-ala^2 cleavage under physiological circumstances. However, this modification does not provide longer period before the inactivation by the enzyme than 4 hours which is insufficient for the treatment. The encapsulation of D-ala^2-GLP-1, similar to the one of long acting exenatide, allowed D-ala^2-GLP-1 to survive in gastrointestinal tract and does not alter its the effect in any other way than prolonging the time before its cleavage by DPP-4. The microspheres consisted of 48 % poly(lactide-co-glycolide)-COOH, 50 % olive oil used as a filler and 2 % of the peptide. This method provided 10 h duration of action suggesting an optimum of twice-daily dosing [98]. Another GLP-1 mimetic that can be given orally is receptor agonist BMS-686117, an inhalable, spray-dried powder formulation. Rats received the agent intratrachelly. BMS-686117 maintained 45 % bioavailability and was absorbed more rapidly in comparison to relative subcutaneous administration. It was detected in plasma for minimally 6 hours after. The agent proved itself to be useful in controlling glucose homeostasis [99]. Molecule with availability to be administered orally and possess antidiabetic effects is Boc5. Boc5 is substituted cyclobutan and the first GLP-1 agonist of non-peptidic origin which allows it to pass the gut without being degraded. The dose of 3 mg each day given to *db/db* mice revealed its ability to reduce HbA_{1C}, improve glucose tolerance, slow down gastric emptying and promote weight loss. After 5 weeks, the levels of HbA_{1C} were the same for *db/db* mice as for controls and continued to be so up to 10 weeks subsequent the end of the study [99]. Targeting some of the G-protein coupled receptor (GPR) on endocrine cells provided enhance GIP and GLP-1 secretion. An agonist of GPR119 was able to augment the concentration of cAMP in GLUTag cells. *In vivo* it also increased GLP-1 levels, mainly when combined with a DPP-4 inhibitor. Moreover, it promoted increase in GIP as well. Reduction of glucose plasma concentration ranged from 42 to 60 % in treated mice. In GPR119-deficient mice or by GPR119 blockade the effect diminished [37].

Incretin-based Therapies for Obesity Treatment

GLP-1 is known for its reducing effect on food intake by regulation of appetite and energy expenditure via hypothalamus, important region of the brain responsible number of metabolic processes [64]. Together with the fact that GLP-1 slows gastric emptying [57] it indicates possible suitability of GLP-1 for inducing weight loss on a long-term basis.

Pathophysiology of Obesity

Causes of obesity are multifactorial and may involve eating disorders, impaired levels of hormones, imbalanced neural circuits and most frequently a long-term excess of energy intake over energy expenditure in combination with genetic component [100]. Several studies suggest that incretin response is impaired in obese in comparison with lean healthy subjects (based on insulin, C-peptide or insulin release). Insulin levels tend to be higher in obese patients as well as secretory responses of β cells. Glucagon levels are also increased, suggesting that hyperglucagonaemia may play a role in the early pathophysiology of altered glucose control [101]. Decreased incretin response might be a consequence of dysregulated concentrations of incretin hormones and additionally perhaps loss of its insulinotropic efficacy. However, GLP-1 levels in obese subjects tend to vary. In some studies, postprandial GLP-1 levels were lower, whereas fasting plasma concentration did not differ between obese and healthy subjects [102]. While other studies came to the conclusion that postprandial GLP-1 response is inversely proportional to BMI and glucose tolerance in independent, additive manner [103]. Nonetheless, some studies have not found any alteration in GLP-1 except for mean baseline values [101]. Whether the impairment is rather a cause or consequence of obesity is still unclear. One of the reports suggests disruption in response of L-cells to carbohydrates and decreased secretion of GLP-1 as a result. Glucose disposal and increased plasma insulin concentrations also occurred [104]. As reduced incretin effect does not seem to correlate with GLP-1 levels in some of the cases, reduced ability of β-cells to respond might be the key. The cause is most likely insulin resistance and lipotoxicity that accompanies the condition of obesity [101].

Obese subjects tend to evaluate their hunger as higher in comparison with obese subjects after weight loss. Subjects after weight reduction even often report their feeling of satiety as higher after the same meal as they were given before when they were obese [102].

Given the influence of GLP-1 on food intake and blood glucose control, its dysregulation could lead to a cycle in which weight gain leads to incretin impairment, hyperglycaemia, further weight gain and so on.

Possible Link from Obesity to Type 2 Diabetes Mellitus

Because of the fact that incretin impairment and hyperglucagonaemia are both present in obesity and T2DM patients, questions about possible linkage between these two diseases are being discussed. T2DM in obese patients might be developed as a result of alterations in β-cell mass consequent to insulin resistance and lipotoxicity and additionally by progressive glucotoxicity [101]. Some studies suggest that because obesity often precedes T2DM, incretin impairment could also precede it [103]. However, other theories explain the defect as a secondary dysfunction. The latter is supported by the fact that normalization of blood glucose using insulin infusion does not increase postprandial GLP-1 nor GIP concentration but improve β-cell responsiveness [105].

Incretin-based Therapies for Obesity

Endogenous GLP-1 promotes satiety [102] after food intake and delays gastric emptying [57] and therefore leads to decreased postprandial glucose excursions and reduced intake at the following meal. If the effect is to be mimicked by pharmacological agents, it could result in weight loss and thus be suitable for obesity treatment. However, incretin-based treatment is approved for plasma glucose control in T2DM patients only, so far.

It is well known in addition to the effect of lowering levels of plasma glucose, incretins promote reductions in body weight as evidenced in clinical trials. In a meta-analysis of incretin based therapies, mean weight loss with GLP-1 mimetics account for approximate reduction of -2.0 kg and DPP-4 inhibitors of -0.2 to -0.6 kg. Therefore, it is clear that GLP-1 mimetics had significant effect on weight loss, whereas DPP-4 inhibitors, often described as having neutral effects, were linked only to a trend towards decreasing body weight. Mean [95 % CI] changes in kg were: exenatide twice daily −1.94 [−2.35 to −1.53]; exenatide once weekly, −2.41 [−2.83 to −1.99]; liraglutide once daily, −1.66 [−2.43 to −0.88]; alogliptin, −0.27 [−0.87 to −0.34]; saxagliptin, −0.64 [−1.11 to −0.16]; sitagliptin, −0.29 [−0.61 to +0.03]; and vildagliptin, −0.21 [−0.84 to +0.42] (Figure 4) [93].

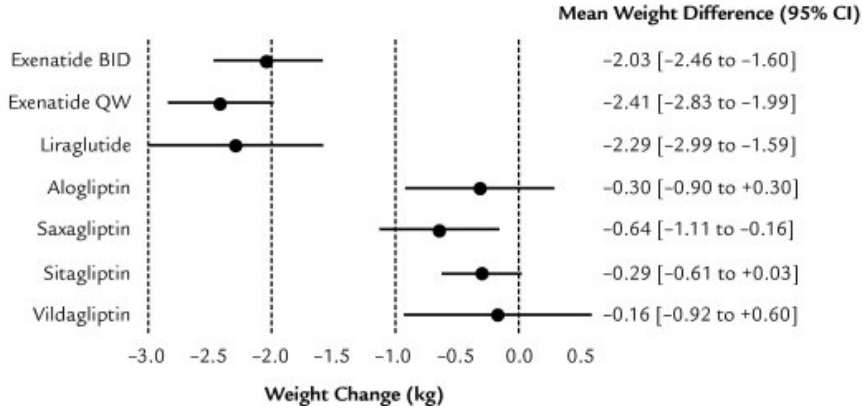

Figure 4 Overall mean changes from baseline in weight with the use of glucagon-like peptide 1 receptor agonists or DPP-4 inhibitors at the highest maintenance doses evaluated. Adapted from Aroda VR, Henry RR, Han J, Huang W, DeYoung MB, Darsow T, Hoogwerf BJ: *Efficacy of GLP-1 receptor agonists and DPP-4 inhibitors: meta-analysis and systematic review*. Clin Ther 2012, 34(6):1247-1258.e1222.

A study examined the effects of exenatide 10 μg twice daily alongside lifestyle modifications in obese subjects (BMI 39.6 ± 7.0 kg/m^2) controlled by placebo. After 24 weeks, weight of subjects on exenatide dropped by 5.1 ± 0.5 kg. Control subjects showed reduction of 1.6 ± 0.5 kg. After another four weeks, subsequent the end of the study, the weight loss was mostly sustained with increase in 0.5 kg only in both groups. A greater percentage of patients receiving exenatide exhibited weight loss of ≥5 % body weight when compared with placebo (32 vs. 17 %, respectively). Subjects, who did not report nausea experienced greater weight reduction (−4.1 ± 0.8 kg vs subjects with nausea −3.8 ± 1.2 kg). Food intake was significantly lower in the exenatide-treated group compared with the placebo-treated group [106].

Liraglutide was proved to be efficacious and well tolerated as a weight-loss treatment in a phase 2 trial that lasted for 2 years. The study consisted of a 2-week placebo period then 20-week main trial period followed, after which subjects could consent to enter the extension period. Subsequently, subjects received randomized treatment until the end of year 1, afterward subjects were moved to liraglutide 2.4 mg group, with a dose escalation to 3.0 mg until the study end. Mean weight loss in between screening and randomization was 1.3 ± 1.4 kg in all groups. Subjects receiving 3.0 mg liraglutide for 2 years experienced weight reduction of 10.3 ± 7.1 kg. Those on liraglutide 2.4 mg at the beginning, who switched to the dose of 3.0 mg, showed an estimated weight loss of 7.8 kg. Nearly 70 % of 2.4/3.0 mg recipients maintained reduction >5 % of screening weight at year 2. 43 % maintained >10 % decrease and 25 % maintained >15 % decrease. Seven subjects developed antibodies to liraglutide over the 2-year trial period. Quality of life improved in all groups [107].

The subcutaneous mode of administration of GLP-1 mimetics may be a barrier to some individuals, but available reports from patients that participated in clinical trials suggest that its clinical benefits outweigh the barriers associated with injections [108]. Incretin mimetics provide only a moderate change to body weight (up to approximately 7.8 kg) but in comparison with currently prescribed orlistat, the change is significantly greater with GLP-1 analogues [107].

One of the approaches under the development is a vaccination against GIP. $GIP^{-/-}$ mice were resistant to diet-induced obesity. Thus, inhibition of the impact of GIP might be a possible approach in obesity treatment. GIP peptides attached to virus-like particles were injected and promoted high titers of specific antibodies against GIP (85 % vs. 4 % in control mice). The strong antibody response led to 35 % reduction of body weight from the baseline. DEXA scan showed reduction only in body fat, while the lean mass stayed unaffected. The reason for weight loss was higher rate of basal metabolism and energy

expenditure. Glucose homeostasis remained unaffected. No evidence of inflammation in the gut was observed in histological sections. The potential role of GIP in development of obesity in man need to be further investigated but research on vaccination against GIP suggest its possible use in obesity treatment [109].

As discussed in previous section targeting the pathophysiology of obesity, the factors leading to its development are very complex and comprise of various regulators of appetite, such as glucagon or GIP for instance. Thus, aiming only at GLP-1 would be anticipated to only have a moderate effect on body weight. Future research on possible combinations of strategies involving more factors affecting weight loss is needed in order to reach greater achievements.

Bariatric Surgery as a Strategy for Treating Type 2 Diabetes and Obesity

Surgical interventions are currently also used in treatment of T2DM and obesity since some of them lead to increase in GLP-1 response and all of them to a significant weight loss. However, the exact mechanism is still unclear. All procedures involve a restrictive component that reduces size of the stomach. The most frequently used, Roux-en-Y gastric bypass (RYGB) prevents the proximal part of small intestine from the contact with ingested food, so that less nutrients are absorbed. In laparoscopic sleeve gastrectomy 75% of the stomach is removed without any intervention on the intestine, leaving just a narrow tube or so-called “sleeve”. Both techniques can be seen in figure 5.

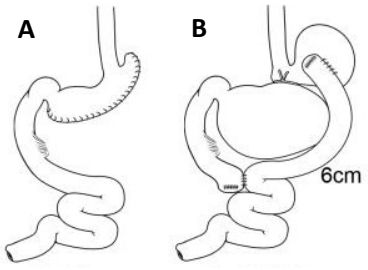

Figure 5 Illustration of (A) sleeve gastrectomy and (B) RYGB. Yin DP, Gao Q, Ma LL, Yan W, Williams PE, McGuinness OP, Wasserman DH, Abumrad NN: *Assessment of different bariatric surgeries in the treatment of obesity and insulin resistance in mice.* Ann Surg 2011, 254(1):73-82.

Values of GLP-1, GIP, insulin, glucagon and β-cell responsiveness were measured in 9 T2DM patients before and after RYGB. Subjects underwent a meal test and received an infusion of either exendin-(9-39)amide or saline. After the surgery glucose tolerance improved, both fasting and postprandial plasma glucose improved. Insulin secretion significantly increased in a response to a meal as well as postprandial GLP-1 and glucagon release. Sensitivity of β-cells doubled. Blockade of GLP-1R action with exendin-(9-39)amide reversed all the improvements to a pre-operative state, suggesting that augmented levels of GLP-1 are very important for the whole process [110]. A retrospective analysis of the clinical data of 50 T2DM patients that underwent RYGB confirmed that HbA_{1C} levels significantly dropped postoperatively and therefore regulation of glucose improved [111].

The RYGB procedure is helpful for obese non-diabetic subjects as well. The surgery leads to similar improvements as in T2DM patients as suggested in a study of 8 of them two weeks after the surgery. Insulin and GLP-1 secretion was significantly enhanced, as well as postprandial glucagon. GIP secretion remained the same as before. The mean weight loss after two weeks was 6.5 ± 0.6 kg, BMI then decreased by 2.2 ± 0.2 kg/m^2 [112]. Changes in secretion of incretins can be achieved by surgical procedures even in cases of obesity of the third degree defined by BMI higher than 40. Thirty-five of these patients were examined after laparoscopic sleeve gastrectomy (LSG) and laparoscopic Roux-en-Y gastric bypass (LRYGB). After the surgery the mean BMI was 32.5 kg/m^2 and 34.4 kg/m^2 in the LSG and LRYGB group, respectively. The average percentage weight lost was 58.8% (%EWL) and the rate of the loss did not differ one year after the surgery. Only one patient diagnosed with T2DM prior the procedure sill remained in diabetic values while in the rest of the patients T2DM was resolved after the surgery. GLP-1 concentration was numerically augmented from 5.6 pmol/l to 6.49 pmol/l but the difference did not reach statistical significance. Additionally, eleven out fifteen patients diagnosed with hypertension showed normal blood pressure postoperatively. The data collected suggest that effective weight loss is reached with LSG and LRYGB procedures and these are thus beneficial for obese as well as third-degree obese patients [113]. However, some patients do not exhibit improvements in weight loss. Study of sixteen good (weight loss of 85.8 ± 4.5 %) and seventeen poor (weight loss of 35.0 ± 1.7 %) responders a year after RYGB procedure was conducted. Those with poor response showed the highest peak of insulin concentration after a fixed meal and also highest value of insulin resistance. The good responders had greater amount of GLP-1 released in response to nutrients. The premeal satiety gradually decreased in good but not poor responders, thus the effectivity of the procedure might rely on gut hormone release that favours the anorectic state [114].

Moreover, Swedish obese subjects study involving 2010 obese subjects after bariatric surgery and 2037 control obese subjects on conventional treatment observed on average for ten to eleven years, claims positive effects of surgery not only on weight loss but also on overall mortality. There were less deaths in the surgery group (101, 5.0%) compared to the control group (129, 6.3%). The hazard ratio based on Cox proportional-hazards regression model adjusted on sex, age and risk factors was 0.71 as compared with the control group. The most common causes of death were of cardiovascular origins and cancer [115]. Another observational study matched patients who had undergone RYGB with identified controls and noticed 58% decrease of relative risk in overall mortality in surgery group in comparison with control subjects. The risk of myocardial infarction and cardiovascular death diminished by 49% and

59%. Five year absolute risks of death were 4% lower when compared with control group (1.8% and 5.8% for the surgery group and controls, respectively) [116].

Available data so far indicate that bariatric surgeries, such as RYGB, LRYGB and LSG, can be significantly beneficial for T2DM and obese patients. Whether these benefits are derived from the weight loss itself, from excessive release of GLP-1 as a result of some types of procedures, changes of metabolism or lifestyle is yet unclear and further research is needed.

Conclusion

Secretion of incretin hormones is a very important factor in preserving normal plasma glucose and insulin concentrations. They are part of a very complex mechanism, involving central nervous system structures, responsible for glucose homeostasis and energy expenditure. However, their secretion and effect are impaired in patients with type 2 diabetes mellitus and obesity. Its ability to promote incretin-induced insulin secretion is reduced and glucose levels, as a result, increased leading to hyperglycaemia. Additionally, β-cell mass reduction could develop. Whether these are causes rather than consequences of diabetes, is a matter of current ongoing research.

With the development of incretin-based therapeutics that preserve its effect for a longer period of time than endogenous incretins, which are cleaved by an enzyme dipeptidyl peptidase-4 in a time range as quick as a few minutes, possible pharmacological use could be investigated. Inhibitors of DPP-4 approaches similar results as incretin-based therapy by different mechanisms. They reduce the effect of DPP-4 and thus increase levels of active GLP-1. In subsequent studies, infusion of incretins proved to possess many beneficial characteristics that make them very promising agents in the treatment of type 2 diabetes mellitus. Exogenous GLP-1 agonists and DPP-4 inhibitors exhibit positive influence on HbA_{1C}, insulin and glucagon plasma levels. GLP-1 receptor agonists also promote food satiety via neuronal circuits, involving hypothalamus and areas of reward system, and thus lead to suppression of caloric intake. Another important response to GLP-1 is the delay of gastric emptying, often accelerated in patients with diabetes, which allows elevation of food satiety. Additionally, incretins are crucial anti-apoptotic and pro-proliferative factors of β-cells and therefore are helpful in maintaining β-cell mass during pancreas transplantations. Incretins have the same therapy potential as other antidiabetic drugs and possibly superior. Although the likelihood of higher risk of cancer development is being debated, no study has verified it so far.

GLP-1 impairment is a typical feature of both obesity and diabetes, and could possibly be part of the connection of the pathophysiology of both conditions. Since GLP-1 agonists result in weight loss, its possible use in treating obesity is being studied intensively. Although the effects are promising, only liraglutide currently belongs to U.S. Food and Drug Administration (FDA) and European Medicine Agency (EMA) approved obesity treatment drugs [117, 118]. GLP-1 receptor agonists seem modify body weight in a desirable way by reducing it and research on GIP antagonism, unlike GIP agonism, also provide evidence of promoting weight loss. On the contrary, DPP-4 inhibitors indicate to be neutral on weight of treated subjects, which is nonetheless still more favourable than other antidiabetic drug that often lead to weight gain. However, achieved weight loss in studies researching incretins is moderate and in most cases does not exceed more than seven to eight kilograms. Lifestyle changes, including dietary modifications along with increase in physical activity, are thus still the most efficient part of obesity management. Incretin-based therapy is thus suggested only as additional method.

In conclusion, incretin hormones play a pivotal role in pathophysiology of type 2 diabetes mellitus and offer a convenient strategy for its treatment. Obesity is also associated with the impairment of incretin secretion which suggest incretin-based therapy use in obesity management. This is also supported by the fact that GLP-1 receptor agonists have a positive effect on weight loss.

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